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### **Supplemental Material**

#### **Urinary Glyphosate, 2,4-D and DEET Biomarkers in Relation to Neurobehavioral Performance in Ecuadorian Adolescents in the ESPINA Cohort**

Briana N.C. Chronister, Kun Yang, Audrey R. Yang, Tuo Lin, Xin M. Tu, Dolores Lopez-Paredes, Harvey Checkoway, Jose Suarez-Torres, Sheila Gahagan, Danilo Martinez, Dana Barr, Raeanne C. Moore, and Jose R. Suarez-Lopez

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**Additional File-** Excel Document

**Table S1.** Change in estimate of adjusted associations of pesticide metabolite concentrations or summary scores with the Attention & Inhibitory Control domain, after adding whether the participant’s mother worked in agriculture while pregnant, or whether the participant’s mother lived with an agricultural worker while pregnant. Participants completed the Secondary Exposures to Pesticides among Children and Adolescents (ESPINA) study July to October 2016 examination that took place in Pedro Moncayo, Ecuador (n=519).

Difference in Attention & Inhibitory Control domain scores per 50% higher biomarker concentration, $\beta$ (95% CI)												
	Original Estimate <sup>a</sup>			Mother Worked in Agriculture While Pregnant <sup>b</sup>			Mother Lived with Agricultural Worker While Pregnant <sup>c</sup>			Both Prenatal Exposures <sup>d</sup>		
	n	$\beta$	95% CI	$\beta$	95% CI	% Change in Estimate	$\beta$	95% CI	% Change in Estimate	$\beta$	95% CI	% Change in Estimate
Herbicide Summary Score	508	-0.090	-0.225, 0.045	-0.091	-0.226, 0.043	0.20%	-0.090	-0.224, 0.045	0.00%	-0.092	-0.227, 0.043	2.22%
2,4-D, observed	507	-0.185	-0.306, 0.064	-0.184	-0.306, 0.063	-0.54%	-0.185	-0.306, 0.064	0.00%	-0.185	-0.306, 0.063	0.00%
2,4-D, imputed	507	0.007	-0.159, 0.174	0.007	-0.159, 0.174	0.00%	0.0075	-0.159, 0.174	7.14%	0.007	-0.159, 0.174	0.00%
Glyphosate	508	0.013	-0.049, 0.075	0.013	-0.049, 0.075	0.00%	0.014	-0.048, 0.076	7.69%	0.013	-0.049, 0.075	0.00%
DEET Summary Score	507	0.008	-0.068, 0.084	0.010	-0.067, 0.086	25.00%	0.008	-0.068, 0.083	0.00%	0.010	-0.066, 0.086	25.00%
ECBA, observed	507	0.013	-0.060, 0.087	0.014	-0.059, 0.087	7.69%	0.013	-0.061, 0.087	0.00%	0.014	-0.060, 0.088	7.69%
ECBA, imputed	507	0.005	-0.048, 0.058	0.005	-0.048, 0.057	0.00%	0.005	-0.048, 0.057	0.00%	0.005	-0.048, 0.058	0.00%
DCBA, observed	507	0.016	-0.039, 0.071	0.016	-0.039, 0.071	0.00%	0.015	-0.040, 0.070	-6.25%	0.016	-0.039, 0.071	0.00%
DCBA, imputed	507	0.007	-0.065, 0.079	0.007	-0.065, 0.079	0.00%	0.007	-0.065, 0.079	0.00%	0.007	-0.065, 0.079	0.00%

<sup>a</sup> Models adjusts for retest learning effect, age, gender, race, creatinine, z-BMI-for-age, monthly salary, and average parental education.

<sup>b</sup> Model adjusts for covariates of model A, except for retest learning effect, and adding whether the participant's mother worked in agriculture while pregnant.

<sup>c</sup> Model adjusts for covariates of model A, except for retest learning effect, and adding whether the participant's mother lived with an agricultural worker while pregnant.

<sup>d</sup> Model adjusts for covariates of model A, except for retest learning effect, and adding whether the participant's mother worked in agriculture while pregnant and whether the participant's mother lived with an agricultural worker while pregnant.

2,4-D, DCBA, and ECBA concentrations below the level of detection (LOD) were imputed using multiple imputation. Imputation using a constant was done for glyphosate.

2,4-D=2,4-dichlorophenoxyacetic acid, DEET = N,N-diethyl-m-toluamide, DCBA = 3-(diethylcarbamoyl) benzoic acid, ECBA = 3-(ethylcarbamoyl) benzoic acid

**Table S2.** Change in estimate of adjusted associations of pesticide metabolite concentrations or summary scores with the Language domain, after adding whether the participant’s mother worked in agriculture while pregnant, or whether the participant’s mother lived with an agricultural worker while pregnant. Participants completed the Secondary Exposures to Pesticides among Children and Adolescents (ESPINA) study July to October 2016 examination that took place in Pedro Moncayo, Ecuador (n=519).

Difference in Language domain scores per 50% higher biomarker concentration, $\beta$ 95% CI												
	Original Estimate <sup>a</sup>			Mother Worked in Agriculture While Pregnant <sup>b</sup>			Mother Lived with Agricultural Worker While Pregnant <sup>c</sup>			Both Prenatal Exposures <sup>d</sup>		
	n	$\beta$	95% CI	$\beta$	95% CI	% Change in Estimate	$\beta$	95% CI	% Change in Estimate	$\beta$	95% CI	% Change in Estimate
Herbicide Summary Score	508	-0.121	-0.246, 0.004	-0.123	-0.249, 0.002	2%	-0.121	-0.246, 0.004	0%	-0.125	-0.25, 0.001	3%
2,4-D, observed	507	-0.127	-0.235, 0.018	-0.126	-0.235, -0.017	-1%	-0.127	-0.235, -0.018	0%	-0.126	-0.235, -0.018	-1%
2,4-D, imputed	507	-0.024	-0.197, 0.148	-0.024	-0.197, 0.148	0%	-0.024	-0.197, 0.149	0%	-0.024	-0.198, 0.149	0%
Glyphosate	508	-0.008	-0.062, 0.046	-0.0085	-0.063, 0.045	6%	-0.008	-0.062, 0.046	0%	-0.0085	-0.063, 0.045	6%
DEET Summary Score	507	0.012	-0.065, 0.090	0.015	-0.063, 0.092	25%	0.012	-0.066, 0.090	0%	0.015	-0.063, 0.093	25%
ECBA, observed	507	-0.024	-0.107, 0.058	-0.024	-0.106, 0.059	0%	-0.025	-0.108, 0.059	4%	-0.023	-0.107, 0.060	-4%
ECBA, imputed	507	0.003	-0.046, 0.052	0.003	-0.046, 0.052	0%	0.003	-0.046, 0.052	0%	0.003	-0.046, 0.052	0%
DCBA, observed	507	-0.01	-0.068, 0.048	-0.009	-0.067, 0.048	-10%	-0.010	-0.068, 0.048	0%	-0.009	-0.066, 0.049	-10%
DCBA, imputed	507	0.007	-0.059, 0.074	0.007	-0.060, 0.074	0%	0.007	-0.059, 0.074	0%	0.007	-0.060, 0.074	0%

<sup>a</sup> Models adjusts for retest learning effect, age, gender, race, creatinine, z-BMI-for-age, monthly salary, and average parental education.

<sup>b</sup> Model adjusts for covariates of model A, except for retest learning effect, and adding whether the participant's mother worked in agriculture while pregnant.

<sup>c</sup> Model adjusts for covariates of model A, except for retest learning effect, and adding whether the participant's mother lived with an agricultural worker while pregnant.

<sup>d</sup> Model adjusts for covariates of model A, except for retest learning effect, and adding whether the participant's mother worked in agriculture while pregnant and whether the participant's mother lived with an agricultural worker while pregnant.

2,4-D, DCBA, and ECBA concentrations below the level of detection (LOD) were imputed using multiple imputation. Imputation using a constant was done for glyphosate.

2,4-D=2,4-dichlorophenoxyacetic acid, DEET = N,N-diethyl-m-toluamide, DCBA = 3-(diethylcarbamoyl) benzoic acid, ECBA = 3-(ethylcarbamoyl) benzoic acid

**Table S3.** Change in estimate of adjusted associations of pesticide metabolite concentrations or summary scores with the Memory & Learning domain, after adding whether the participant’s mother worked in agriculture while pregnant, or whether the participant’s mother lived with an agricultural worker while pregnant. Participants completed the Secondary Exposures to Pesticides among Children and Adolescents (ESPINA) study July to October 2016 examination that took place in Pedro Moncayo, Ecuador (n=519).

Difference in Memory & Learning domain scores per 50% higher biomarker concentration, $\beta$ (95% CI)												
	Original Estimate <sup>a</sup>			Mother Worked in Agriculture While Pregnant <sup>b</sup>			Mother Lived with Agricultural Worker While Pregnant <sup>c</sup>			Both Prenatal Exposures <sup>d</sup>		
	n	$\beta$	95% CI	$\beta$	95% CI	% Change in Estimate	$\beta$	95% CI	% Change in Estimate	$\beta$	95% CI	% Change in Estimate
Herbicide Summary Score	508	-0.123	-0.261, 0.015	-0.125	-0.264, 0.014	2%	-0.123	-0.261, 0.015	0%	-0.126	-0.266, 0.013	2%
2,4-D, observed	507	-0.111	-0.229, 0.008	-0.110	-0.229, 0.008	-1%	-0.111	-0.230, 0.007	0%	-0.111	-0.229, 0.007	0%
2,4-D, imputed	507	0.049	-0.150, 0.248	0.049	-0.150, 0.248	0%	0.049	-0.150, 0.248	0%	0.049	-0.150, 0.248	0%
Glyphosate	508	-0.023	-0.092, 0.047	-0.023	-0.093, 0.046	0%	-0.023	-0.092, 0.046	0%	-0.024	-0.094, 0.046	4%
DEET Summary Score	507	0.012	-0.078, 0.102	0.014	-0.077, 0.106	17%	0.012	-0.078, 0.103	0%	0.015	-0.077, 0.108	25%
ECBA, observed	507	-0.010	-0.10, 0.08	-0.009	-0.102, 0.083	-10%	-0.010	-0.103, 0.083	0%	-0.009	-0.102, 0.085	-10%
ECBA, imputed	507	-0.012	-0.059, 0.074	-0.013	-0.072, 0.047	8%	-0.012	-0.072, 0.047	0%	-0.013	-0.072, 0.047	8%
DCBA, observed	507	0.004	-0.063, 0.071	0.005	-0.062, 0.073	25%	0.004	-0.062, 0.071	0%	0.006	-0.062, 0.074	50%
DCBA, imputed	507	0.012	-0.070, 0.094	0.012	-0.070, 0.093	0%	0.012	-0.07, 0.094	0%	0.011	-0.071, 0.093	-8%

<sup>a</sup> Models adjusts for retest learning effect, age, gender, race, creatinine, z-BMI-for-age, monthly salary, and average parental education.

<sup>b</sup> Model adjusts for covariates of model A, except for retest learning effect, and adding whether the participant's mother worked in agriculture while pregnant.

<sup>c</sup> Model adjusts for covariates of model A, except for retest learning effect, and adding whether the participant's mother lived with an agricultural worker while pregnant.

<sup>d</sup> Model adjusts for covariates of model A, except for retest learning effect, and adding whether the participant's mother worked in agriculture while pregnant and whether the participant's mother lived with an agricultural worker while pregnant.

2,4-D, DCBA, and ECBA concentrations below the level of detection (LOD) were imputed using multiple imputation. Imputation using a constant was done for glyphosate.

2,4-D=2,4-dichlorophenoxyacetic acid, DEET = N,N-diethyl-m-toluamide, DCBA = 3-(diethylcarbamoyl) benzoic acid, ECBA = 3-(ethylcarbamoyl) benzoic acid

**Table S4.** Change in estimate of adjusted associations of pesticide metabolite concentrations or summary scores with the Visuospatial Processing domain, after adding whether the participant’s mother worked in agriculture while pregnant, or whether the participant’s mother lived with an agricultural worker while pregnant. Participants completed the Secondary Exposures to Pesticides among Children and Adolescents (ESPINA) study July to October 2016 examination that took place in Pedro Moncayo, Ecuador (n=519).

Difference in Visuospatial Processing domain scores per 50% higher biomarker concentration, β (95% CI)												
	Original Estimate <sup>a</sup>			Mother Worked in Agriculture While Pregnant <sup>b</sup>			Mother Lived with Agricultural Worker While Pregnant <sup>c</sup>			Both Prenatal Exposures <sup>d</sup>		
	n	β	95% CI	β	95% CI	% Change in Estimate	β	95% CI	% Change in Estimate	β	95% CI	% Change in Estimate
Herbicide Summary Score	508	-0.141	-0.288, 0.006	-0.138	-0.285, 0.009	-2%	-0.145	-0.292, 0.001	3%	-0.143	-0.29, 0.003	1%
2,4-D, observed	507	-0.057	-0.170, 0.055	-0.059	-0.173, 0.054	4%	-0.062	-0.173, 0.049	9%	-0.062	-0.173, 0.049	9%
2,4-D, imputed	507	-0.005	-0.190, 0.181	-0.0046	-0.190, 0.181	-8%	-0.005	-0.190, 0.181	0%	-0.005	-0.190, 0.181	0%
Glyphosate	508	-0.056	-0.136, 0.023	-0.061	-0.141, 0.0018	9%	-0.054	-0.133, 0.026	-4%	-0.057	-0.137, 0.024	2%
DEET Summary Score	5073	-0.06	-0.14, 0.02	-0.06	-0.14, 0.02	0%	-0.05	-0.13, 0.03	-17%	-0.06	-0.14, 0.02	0%
ECBA, observed	507	-0.009	-0.09, 0.07	-0.011	-0.088, 0.067	22%	-0.006	-0.084, 0.072	-33%	-0.007	-0.085, 0.071	-22%
ECBA, imputed	507	0.0001	-0.06, 0.06	0.0004	-0.06, 0.06	300%	0.0004	-0.057, 0.058	300%	0.0004	-0.057, 0.058	300%
DCBA, observed	507	-0.03	-0.087, 0.026	-0.032	-0.088, 0.024	7%	-0.028	-0.085, 0.028	-7%	-0.029	-0.086, 0.027	-3%
DCBA, imputed	507	0.016	-0.062, 0.094	0.016	-0.062, 0.094	0%	0.015	-0.063, 0.093	-6%	0.016	-0.062, 0.094	0%

<sup>a</sup> Models adjusts for retest learning effect, age, gender, race, creatinine, z-BMI-for-age, monthly salary, and average parental education.

<sup>b</sup> Model adjusts for covariates of model A, except for retest learning effect, and adding whether the participant's mother worked in agriculture while pregnant.

<sup>c</sup> Model adjusts for covariates of model A, except for retest learning effect, and adding whether the participant's mother lived with an agricultural worker while pregnant.

<sup>d</sup> Model adjusts for covariates of model A, except for retest learning effect, and adding whether the participant's mother worked in agriculture while pregnant and whether the participant's mother lived with an agricultural worker while pregnant.

Concentrations below the level of detection (LOD) for 2,4-D, DCBA, and ECBA were imputed using multiple imputation. Imputation using a constant (LOD/√2) was done for glyphosate.

2,4-D=2,4-dichlorophenoxyacetic acid, DEET = N,N-diethyl-m-toluamide, DCBA = 3-(diethylcarbamoyl) benzoic acid, ECBA = 3-(ethylcarbamoyl) benzoic acid

**Table S5.** Change in estimate of adjusted associations of pesticide metabolite concentrations or summary scores with the Social Perception domain, after adding whether the participant’s mother worked in agriculture while pregnant, or whether the participant’s mother lived with an agricultural worker while pregnant. Participants completed the Secondary Exposures to Pesticides among Children and Adolescents (ESPINA) study July to October 2016 examination that took place in Pedro Moncayo, Ecuador (n=519).

Difference in Social Perception domain scores per 50% higher biomarker concentration, $\beta$ (95% CI)												
	Original Estimate <sup>a</sup>			Mother Worked in Agriculture While Pregnant <sup>b</sup>			Mother Lived with Agricultural Worker While Pregnant <sup>c</sup>			Both Prenatal Exposures <sup>d</sup>		
	n	$\beta$	95% CI	$\beta$	95% CI	% Change in Estimate	$\beta$	95% CI	% Change in Estimate	$\beta$	95% CI	% Change in Estimate
Herbicide Summary Score	508	-0.240	-0.383, -0.097	-0.246	-0.388, -0.104	3%	-0.235	-0.378, -0.092	-2%	-0.242	-0.385, -0.098	1%
2,4-D, observed	507	-0.099	-0.263, 0.066	-0.095	-0.261, 0.070	-4%	-0.093	-0.254, 0.069	-6%	-0.092	-0.255, 0.071	-7%
2,4-D, imputed	507	0.026	-0.172, 0.225	0.026	-0.173, 0.225	0%	0.026	-0.171, 0.224	0%	0.026	-0.172, 0.224	0%
Glyphosate	508	-0.077	-0.138, 0.017	-0.08	-0.139, 0.020	4%	-0.075	-0.136, 0.014	-3%	-0.078	-0.138, 0.018	1%
DEET Summary Score	507	-0.004	-0.105, 0.097	0.003	-0.098, 0.103	-175%	-0.008	-0.108, 0.093	100%	-0.002	-0.103, 0.099	-50%
ECBA, observed	507	-0.040	-0.130, 0.051	-0.037	-0.127, 0.053	-8%	-0.044	-0.135, 0.047	10%	-0.041	-0.132, 0.050	3%
ECBA, imputed	507	-0.001	-0.059, 0.057	-0.001	-0.059, 0.057	0%	-0.001	-0.059, 0.056	0%	-0.002	-0.060, 0.056	100%
DCBA, observed	507	-0.021	-0.091, 0.049	-0.018	-0.088, 0.052	-14%	-0.024	-0.094, 0.046	14%	-0.021	-0.092, 0.049	0%
DCBA, imputed	507	0.018	-0.06, 0.097	0.017	-0.062, 0.096	-6%	0.019	-0.060, 0.097	6%	0.018	-0.061, 0.097	0%

<sup>a</sup> Models adjusts for retest learning effect, age, gender, race, creatinine, z-BMI-for-age, monthly salary, and average parental education.

<sup>b</sup> Model adjusts for covariates of model A, except for retest learning effect, and adding whether the participant's mother worked in agriculture while pregnant.

<sup>c</sup> Model adjusts for covariates of model A, except for retest learning effect, and adding whether the participant's mother lived with an agricultural worker while pregnant.

<sup>d</sup> Model adjusts for covariates of model A, except for retest learning effect, and adding whether the participant's mother worked in agriculture while pregnant and whether the participant's mother lived with an agricultural worker while pregnant.

Concentrations below the level of detection (LOD) for 2,4-D, DCBA, and ECBA were imputed using multiple imputation. Imputation using a constant (LOD/ $\sqrt{2}$ ) was done for glyphosate

2,4-D=2,4-dichlorophenoxyacetic acid, DEET = N,N-diethyl-m-toluamide, DCBA = 3-(diethylcarbamoyl) benzoic acid, ECBA = 3-(ethylcarbamoyl) benzoic acid

**Table S6.** Pearson correlation (r [p-value]) matrix of urinary pesticide biomarker concentrations of participants of the Secondary Exposures to Pesticides among Children and Adolescents (ESPINA) study July to October 2016 examination that took place in Pedro Moncayo, Ecuador (n=519).

	<b>2,4-D</b>	<b>Glyphosate</b>	<b>ECBA</b>	<b>DCBA</b>	<b>AND</b>	<b>PNP</b>	<b>TCPy</b>	<b>3-PBA</b>
<b>2,4-D</b>	1	0.13 (0.003)	-0.01 (0.87)	0.004 (0.92)	-0.01 (0.74)	0.03 (0.44)	0.02 (0.61)	0.00 (0.92)
<b>Glyphosate</b>	-	1	-0.01 (0.80)	-0.01 (0.79)	0.001 (0.98)	0.28 ( $<0.0001$ )	0.32 ( $<0.0001$ )	0.16 ( $<0.01$ )
<b>ECBA</b>	-	-	1	0.99 ( $<0.001$ )	-0.01 (0.83)	-0.03 (0.47)	-0.01 (0.81)	0.13 (0.004)
<b>DCBA</b>	-	-	-	1	-0.01 (0.79)	-0.03 (0.48)	-0.01 (0.88)	0.13 (0.003)
<b>AND</b>	-	-	-	-	1	0.15 (0.001)	0.03 (0.49)	0.06 (0.17)
<b>PNP</b>	-	-	-	-	-	1	0.30 ( $<0.0001$ )	0.20 ( $<0.0001$ )
<b>TCPy</b>	-	-	-	-	-	-	1	0.44 ( $<0.0001$ )
<b>3-PBA</b>	-	-	-	-	-	-	-	1

The p-values was calculated by taking calculating the Pearson correlation coefficient between two biomarkers. A p-value ( $p<0.05$ ) indicates that there is a statistically significant correlation between the tested pair of biomarkers.

2,4-D=2,4-dichlorophenoxyacetic acid, DEET = N,N-diethyl-m-toluamide, DCBA = 3-(diethylcarbamoyl) benzoic acid, ECBA = 3-(ethylcarbamoyl) benzoic acid, AND=Acetamiprid-N-desmethyl, PNP=para-Nitrophenol, TCPy=3,5,6-Trichloro-2-pyridinol, 3-PBA=3-phenoxybenzoic acid`



**Table S7.** Curvilinear results of generalized estimating equations (GEE) of associations between pesticide exposure and the five NEPSY-II domains for participants of the Secondary Exposures to Pesticides among Children and Adolescents (ESPINA) study July to October 2016 examination that took place in Pedro Moncayo, Ecuador (n=519).

		Attention & Inhibitory Control <sup>a</sup>		Language <sup>b</sup>		Memory & Learning <sup>b</sup>		Visuospatial Processing <sup>b</sup>		Social Perception <sup>b</sup>	
	n	β <sup>2</sup> (95%CI), p-value	β (95%CI), p-value	β <sup>2</sup> (95%CI), p-value	β (95%CI), p-value	β <sup>2</sup> (95%CI), p-value	β (95%CI), p-value	β <sup>2</sup> (95%CI), p-value	β (95%CI), p-value	β <sup>2</sup> (95%CI), p-value	β (95%CI), p-value
Herbicide Summary Score	519	-0.38 (-0.71, -0.05), 0.02	-0.32 (-0.65, 0.01), 0.06	-0.19 (-0.48, 0.10), 0.20	-0.40 (-0.70, -0.10), 0.01	-0.13 (-0.44, 0.18), 0.40	-0.36 (-0.69, -0.02), 0.04	-0.05 (-0.38, 0.28), 0.77	-0.35 (-0.69, -0.01), 0.05	0.20 (-0.14, 0.54), 0.25	-0.57 (-0.93, -0.21),0.001
2,4-D, observed	518	-0.04 (-0.18, 0.11), 0.64	-0.48 (-0.79, -0.17), 0.001	0.05 (-0.05, 0.14), 0.37	-0.32 (-0.57, -0.07), 0.01	-0.05 (-0.17, 0.06), 0.35	-0.30 (-0.56, -0.03), 0.03	-0.10 (-0.23, 0.02), 0.11	-0.18 (-0.42, 0.06), 0.15	0.21 (0.09, 0.34),<0.001	-0.21 (-0.47, 0.05), 0.11
2,4-D, imputed	518	0.02 (-0.39, 0.44), 0.92	0.06 (-0.97, 1.09), 0.91	0.01 (-0.42, 0.44), 0.96	-0.08 (-1.17, 1.01), 0.89	0.01 (-0.49, 0.51), 0.97	-0.19 (-1.07, 1.44), 0.77	0.01 (-0.46, 0.48), 0.96	0.05 (-1.12, 1.21), 0.94	0.03 (-0.47, 0.53), 0.90	0.11 (-1.14, 1.37), 0.86
Glyphosate	519	-0.03 (-0.08, 0.01), 0.12	-0.08 (-0.29, 0.13), 0.47	-0.02 (-0.05, 0.02), 0.36	-0.11 (-0.30, 0.07), 0.23	-0.02 (-0.07, 0.03), 0.40	-0.13 (-0.34, 0.07), 0.21	-0.03 (-0.07, 0.02), 0.29	-0.14 (-0.35, 0.07), 0.21	-0.00 (-0.05, 0.04), 0.87	-0.21 (-0.43, 0.00), 0.05
DEET Summary Score	518	0.03 (-0.17, 0.22), 0.77	0.03 (-0.23, 0.30), 0.81	-0.12 (-0.33, 0.10), 0.28	-0.05 (-0.36, 0.26), 0.75	-0.03 (-0.25, 0.20), 0.80	0.02 (-0.31, 0.36), 0.88	0.04 (-0.16, 0.25), 0.68	-0.10 (-0.37, 0.17), 0.46	-0.13 (-0.35, 0.09), 0.24	-0.09 (-0.44, 0.26), 0.61
ECBA, observed	518	-0.05 (-0.12, 0.03), 0.22	0.14 (-0.12, 0.39), 0.30	0.04 (-0.05, 0.13), 0.39	-0.14 (-0.42, 0.14), 0.33	0.03 (-0.05, 0.12), 0.46	-0.09 (-0.41, 0.23), 0.58	0.03 (-0.04, 0.11), 0.37	0.03 (-0.04, 0.11), 0.37	0.01 (-0.08, 0.10), 0.79	0.01 (-0.08, 0.10), 0.79
ECBA, imputed	518	0.00 (-0.05, 0.05), 0.95	0.01 (-0.12, 0.14), 0.85	0.01 (-0.04, 0.05), 0.80	0.00 (-0.12, 0.13), 0.96	0.00 (-0.06, 0.06), 0.94	-0.03 (-0.18, 0.12), 0.70	0.00 (-0.06, 0.06), 0.98	0.00 (-0.06, 0.06), 0.98	0.01 (-0.05, 0.07), 0.80	0.01 (-0.05, 0.07), 0.80
DCBA, observed	518	-0.02 (-0.08, 0.03), 0.40	0.08 (-0.12, 0.28), 0.42	0.01 (-0.05, 0.07), 0.71	-0.03 (-0.22, 0.16), 0.76	0.02 (-0.04, 0.09), 0.44	0.03 (-0.17, 0.23), 0.75	0.04 (-0.01, 0.10), 0.14	-0.20 (-0.42, 0.02), 0.08	0.01 (-0.05, 0.08), 0.67	-0.08 (-0.33, 0.18), 0.56
DCBA, imputed	518	0.00 (-0.07, 0.07), 0.99	0.01 (-0.16, 0.19), 0.87	-0.01 (-0.07, 0.06), 0.87	0.02 (-0.15, 0.18), 0.84	-0.01 (-0.09, 0.07), 0.83	0.04 (-0.42, 0.51), 0.86	-0.00 (-0.08, 0.07), 0.91	0.02 (-0.16, 0.21), 0.80	-0.01 (-0.09, 0.07), 0.82	0.03 (-0.16, 0.23), 0.73

<sup>a</sup>Model adjusts for retest learning effect, age, gender, race, creatinine, z-BMI-for-age, monthly salary, and average parental education.

<sup>b</sup>Model adjusts for covariates of model A, minus retest learning effect.

The p-value was obtained by running a generalized estimating equation with a curvilinear (e.g. 2,4-D\*2,4-D) and linear biomarker (e.g. 2,4-D) variable in the same model, adjusting for confounders. A p-value<0.05 for the curvilinear term indicates the presence of curvilinearity.

2 Concentrations below the level of detection (LOD) for 2,4-D, DCBA, and ECBA were imputed using multiple imputation. Imputation using a constant (LOD/√2) was done for glyphosate

2,4-D=2,4-dichlorophenoxyacetic acid, DEET = N,N-diethyl-m-toluamide, DCBA = 3-(diethylcarbamoyl) benzoic acid, ECBA = 3-(ethylcarbamoyl) benzoic acid

**Table S8.** Interaction terms between gender and herbicide or DEET metabolite concentrations or summary scores with neurobehavioral domain scores for participants of the Secondary Exposures to Pesticides among Children and Adolescents (ESPINA) study July to October 2016 examination that took place in Pedro Moncayo, Ecuador (n=519).

Difference in neurobehavioral domain score per 50% higher metabolite concentration or summary score						
	n	Attention and Inhibitory Control <sup>a</sup>	Language <sup>b</sup>	Memory and Learning <sup>b</sup>	Visuospatial Processing <sup>b</sup>	Social Perception <sup>b</sup>
<b>Herbicide Summary Score</b>	519	0.29 (-0.29, 0.88)	0.12 (-0.45, 0.69)	0.28 (-0.32, 0.88)	0.31 (-0.34, 0.97)	-0.03 (-0.65, 0.59)
<b>2,4-D, observed</b>	518	-0.13 (-0.64, 0.39)	-0.17 (-0.67, 0.33)	0.24 (-0.30, 0.77)	0.40 (-0.11, 0.91)	0.11 (-0.55, 0.78)
<b>2,4-D, imputed</b>	519	0.23 (-0.35, 0.80)	0.06 (-0.55, 0.67)	0.23 (-0.45, 0.90)	0.40 (-0.25, 1.05)	-0.12 (-0.83, 0.59)
<b>Glyphosate</b>	518	0.07 (-0.22, 0.35)	-0.02 (-0.28, 0.24)	0.07 (-0.25, 0.38)	0.12 (-0.23, 0.47)	-0.02 (-0.29, 0.25)
<b>DEET Summary Score</b>	518	0.04 (-0.30, 0.38)	0.07 (-0.30, 0.44)	-0.30 (-0.69, 0.10)	0.20 (-0.18, 0.58)	-0.06 (-0.53, 0.40)
<b>DCBA, observed</b>	518	0.00 (-0.27, 0.27)	-0.01 (-0.36, 0.34)	-0.30 (-0.64, 0.03)	0.16 (-0.13, 0.45)	-0.09 (-0.48, 0.30)
<b>DCBA, imputed</b>	518	-0.02 (-0.36, 0.33)	0.01 (-0.32, 0.33)	0.00 (-0.38, 0.39)	0.08 (-0.28, 0.44)	0.02 (-0.36, 0.40)
<b>ECBA, observed</b>	518	0.05 (-0.30, 0.40)	0.01 (-0.51, 0.53)	-0.27 (-0.74, 0.20)	-0.02 (-0.20, 0.16)	-0.08 (-0.64, 0.49)
<b>ECBA, imputed</b>	518	-0.02 (-0.27, 0.23)	-0.04 (-0.28, 0.20)	0.02 (-0.26, 0.30)	0.25 (-0.19, 0.69)	-0.05 (-0.33, 0.23)

<sup>a</sup> Model adjusts for retest learning effect, age, gender, race, creatinine, z-BMI-for-age, monthly salary, and average parental education.

<sup>b</sup> Model adjusts for covariates of model A except for retest learning effect.

Concentrations below the level of detection (LOD) for 2,4-D, DCBA, and ECBA were imputed using multiple imputation. Imputation using a constant (LOD/ $\sqrt{2}$ ) was done for glyphosate

2,4-D=2,4-Dichlorophenoxyacetic acid, DCBA=3-(diethylcarbamoyl) benzoic acid, ECBA= 3-(ethylcarbamoyl) benzoic acid.

**Table S9.** Mediation analyses of testosterone on the relationship between each pesticide metabolite or summary score and cognitive domains using structural equation modeling in participants of the July to October 2016 Secondary Exposures to Pesticides among Children and Adolescents (ESPINA) study examination (N=519) based in Pedro Moncayo, Ecuador. Statistically significant associations reflect the presence of mediation.

	n	Testosterone (n=512)	n	Estradiol (n=247)	n	Cortisol (n=515)	n	DHEA (n=495)
<b>Herbicide Summary Score</b>								
<b>Attention and Inhibitory Control<sup>a</sup></b>	512	0.018 (-0.010, 0.046)	247	0.008 (-0.023, 0.039)	515	-0.003 (-0.023, 0.017)	495	0.008 (-0.011, 0.026)
<b>Language<sup>b</sup></b>	512	0.028 (-0.006, 0.06)	247	0.015 (-.023, 0.052)	515	-0.004 (-0.025, 0.018)	495	-0.003 (-0.019, 0.013)
<b>Memory and Learning<sup>b</sup></b>	512	0.009 (-0.018, 0.035)	247	-0.001 (-0.036, 0.034)	515	-0.005 (-0.034, 0.025)	495	-0.009 (-0.031, 0.013)
<b>Visuospatial Processing<sup>b</sup></b>	512	-0.006 (-0.032, 0.019)	247	0.004 (-0.02, 0.04)	515	0.00 (-0.004, 0.004)	495	-0.011 (-0.033, 0.012)
<b>Social Perception<sup>b</sup></b>	512	0.024 (-0.01, 0.058)	247	-0.015 (-0.056, 0.026)	515	-0.001 (-0.009, 0.007)	495	-0.001 (-0.009, 0.007)
<b>Glyphosate</b>								
<b>Social Perception<sup>b</sup></b>	512	0.021 (-0.005, 0.046)	247	-0.011 (-0.037, 0.016)	515	0.00 (-0.005, 0.004)	495	0.00 (-0.008, 0.007)
<b>2,4-D</b>								
<b>Attention and Inhibitory Control<sup>a</sup></b>	511	0.005 (-0.013, 0.023)	247	0.008 (-0.026, 0.041)	514	-0.003 (-0.034, 0.028)	494	0.00 (-0.002, 0.002)
<b>Language<sup>b</sup></b>	511	0.013 (-0.029, 0.055)	247	-0.003 (-0.031, 0.026)	514	-0.002 (-0.027, 0.022)	494	0.00 (-0.005, 0.005)
<b>Memory and Learning<sup>b</sup></b>	511	0.013 (-0.028, 0.054)	247	0.008 (-0.030, 0.046)	514	-0.004 (-0.044, 0.036)	494	-0.001 (-0.024, 0.022)

<sup>a</sup>Model adjusts for retest learning effect, age, gender, race, creatinine, z-BMI-for-age, monthly salary, and average parental education.

<sup>b</sup>Model adjusts for covariates of model A except for retest learning effect.

β (95%CI)=indirect effect of hormone on the neurobehavioral domain

2,4-D, DCBA, and ECBA concentrations below the level of detection (LOD) were imputed using multiple imputation. Imputation using a constant was done for glyphosate.

2.4-D=2,4-Dichlorophenoxyacetic acid, DCBA=3-(diethylcarbamoyl) benzoic acid, ECBA= 3-(ethylcarbamoyl) benzoic acid.

**Supplemental Figure 1.** Directed acyclic graph for the generalized estimated equation models that will assess whether herbicide or DEET biomarker concentration is associated with NEPSY-II domain scores for participants of the Secondary Exposures to Pesticides among Children and Adolescents (ESPINA) study July to October 2016 examination in Pedro Moncayo, Ecuador.

